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Discovery of a Quinoline-4-carboxamide Derivative with a Novel Mechanism of Action, Multistage Antimalarial Activity, and Potent in Vivo Efficacy

Beatriz Baragaña,[†] Neil R. Norcross,[†] Caroline Wilson,[†] Achim Porzelle,[†] Irene Hallyburton,[†] Raffaella Grimaldi,[†] Maria Osuna-Cabello,[†] Suzanne Norval,[†] Jennifer Riley,[†] Laste Stojanovski,[†] Frederick R. C. Simeons,[†] Paul G. Wyatt,[†] Michael J. Delves,[‡] Stephan Meister,[§] Sandra Duffy,^{||} Vicky M. Avery,^{||} Elizabeth A. Winzeler,[§] Robert E. Sinden,[‡] Sergio Wittlin,^{⊥, #} Julie A. Frearson,[†] David W. Gray,[†] Alan H. Fairlamb,[†] David Waterson,[▽] Simon F. Campbell,[▽] Paul Willis,[▽] Kevin D. Read,^{*, †} and Ian H. Gilbert^{*, †}

[†]Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, U.K.

[‡]Cell and Molecular Biology, Department of Life Sciences, Imperial College, London, SW7 2AZ, U.K.

[§]School of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, United States

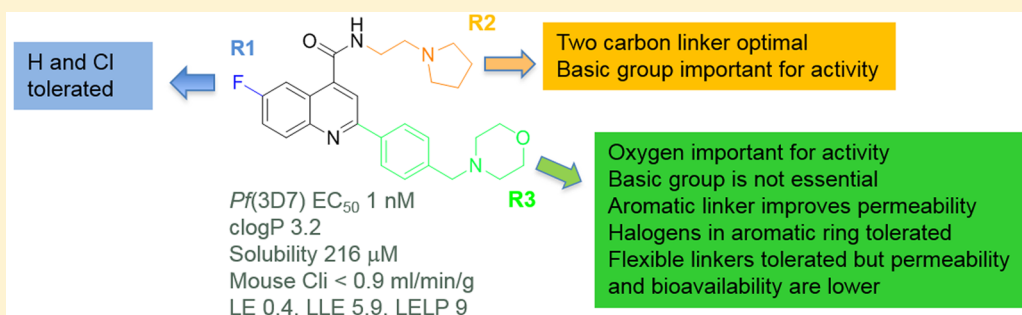
^{||}Eskitis Institute, Griffith University, Brisbane Innovation Park, Nathan Campus, Brisbane, QLD 4111, Australia

[⊥]Swiss Tropical and Public Health Institute, Swiss TPH, Socinstrasse 57, 4051 Basel, Switzerland

[#]University of Basel, CH-4003 Basel, Switzerland

[▽]Medicines for Malaria Venture, International Centre Cointrin, Entrance G, 3rd Floor, Route de Pré-Bois 20, P.O. Box 1826, CH-1215, Geneva 15, Switzerland

Supporting Information



ABSTRACT: The antiparasmodial activity, DMPK properties, and efficacy of a series of quinoline-4-carboxamides are described. This series was identified from a phenotypic screen against the blood stage of *Plasmodium falciparum* (3D7) and displayed moderate potency but with suboptimal physicochemical properties and poor microsomal stability. The screening hit (**1**, EC_{50} = 120 nM) was optimized to lead molecules with low nanomolar in vitro potency. Improvement of the pharmacokinetic profile led to several compounds showing excellent oral efficacy in the *P. berghei* malaria mouse model with ED_{90} values below 1 mg/kg when dosed orally for 4 days. The favorable potency, selectivity, DMPK properties, and efficacy coupled with a novel mechanism of action, inhibition of translation elongation factor 2 (*Pf*EF2), led to progression of **2** (DDD107498) to preclinical development.

INTRODUCTION

Malaria is a devastating disease with over 214 million clinical cases in 2015.¹ In that year alone, the World Health Organization (WHO) estimated 438 000 deaths, mostly among children under five in Sub-Saharan Africa. The current malaria control programs that combine preventive measures with artemisinin combination therapy (ACT) treatment have proven very effective in reducing the malaria burden. Over the

past decade, the number of deaths from malaria has fallen by 4% per year, and between 2000 and 2015 the number of clinical cases of malaria has been estimated to have decreased by 40% where the disease is endemic in Africa.² However, in recent years, parasite resistance to artemisinin has been detected in a

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number of countries in Southeast Asia. For example, in areas along the Cambodia–Thailand border, *Plasmodium falciparum*, the most deadly malaria parasite, has become resistant to most available antimalarial medicines and the spread of multidrug resistance is a major concern.³

The malaria drug discovery portfolio has dramatically improved over the past 10 years.⁴ However, due to the constant battle against drug resistance and to achieve malaria elimination, new chemotypes with novel mechanisms of action are required. New drugs are needed: (1) that are not cross-resistant to existing drugs; (2) that can be given as a single dose; (3) that prevent transmission (active against the sexual stages of the parasite); (4) that can give chemoprotection (active against liver stages). Recently, we reported the discovery and profile of **2** (DDD107498),⁵ a quinoline-4-carboxamide with excellent pharmacokinetic and antimalarial properties, including activity against multiple life-cycle stages of the parasite. Moreover, this compound acts through a novel mechanism of action for antimalarial chemotherapy, inhibition of translation elongation factor 2 (*Pf*EF2), which is critical for protein synthesis.⁵ In this paper we describe the medicinal chemistry program that led to the discovery of **2**.

The quinoline-4-carboxamide series was identified from a phenotypic screen of the Dundee protein kinase library⁶ against the blood stage of the *P. falciparum* 3D7 strain. The most active compound of the original hit series displayed good activity in vitro against a chloroquine sensitive *P. falciparum* strain (3D7) and good selectivity index (>100-fold) against a human cell line (MRC-5). However, hit compound **1** had a high clogP and poor aqueous solubility and was metabolically unstable with a high hepatic microsomal intrinsic clearance (C_{li}). (Figure 1 and Table 1).

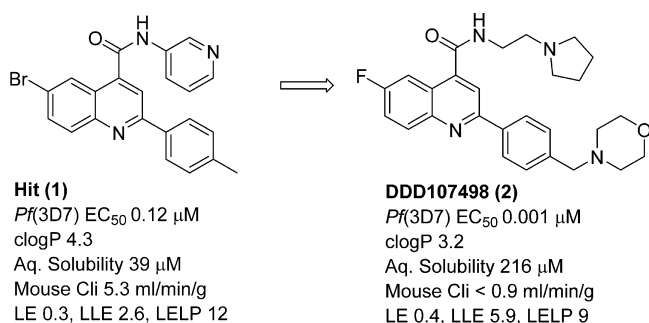


Figure 1. Key data for screening hit **1** and preclinical candidate **2**. Data reported previously.⁵

RESULTS AND DISCUSSION

The initial aim of the hit to lead program was to improve potency (*Pf* EC₅₀(3D7) < 0.1 μM), aqueous solubility (>100 μM), and metabolic stability (mouse liver microsomes C_{li} < 5 mL min⁻¹ g⁻¹) of compound **1**. Iterative rounds of drug design, synthesis, and biological evaluation were driven by the Medicines for Malaria Venture (MMV) compound progression criteria.⁷ Initial modifications were directed toward improving the physicochemical properties particularly reducing lipophilicity. The clogP of the hit was 4.3, which is higher than average for oral drugs and may contribute to the poor aqueous solubility and hepatic microsomal instability.⁸ Several points for modification on the scaffold were identified that could address the high lipophilicity: the bromine atom (R¹) significantly adds

to lipophilicity, as do aromatic substituents in the carboxamide (R²) and quinoline (R³) moieties. High numbers of aromatic rings are associated with unfavorable lipophilicity and poor compound developability.⁹

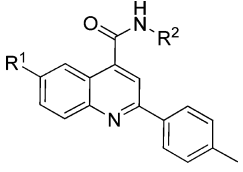
The initial focus was on the R¹ and R² substituents. Quinoline-4-carboxamides **10–19** were prepared in two steps from the corresponding isatin (Scheme 1), employing the Pfitzinger reaction with 1-(*p*-tolyl)ethanone using potassium hydroxide as a base in a mixture of ethanol and water at 125 °C under microwave irradiation to afford the quinoline-4-carboxylic acid **3**.¹⁰ Coupling of **3** with the corresponding amine, using EDC and HOBt in DMF, led to compounds **10–19** (Scheme 1).

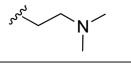
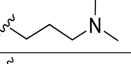
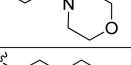
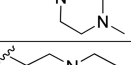
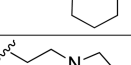
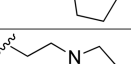
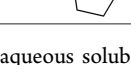
Replacement of the bromine moiety at R¹ with chlorine (**10**) or fluorine (**11**)⁵ was tolerated without significant loss of activity while decreasing molecular weight and clogP (Table 1). However, removal of the halogen altogether in compound **12** led to an 8-fold drop in potency. Although fluorinated compound **11** was less lipophilic than initial hit **1**, it still showed poor solubility and metabolic stability.

On the basis of these results, we next turned to the R² position with the aim of reducing the number of aromatic rings. A range of nonaromatic amines able to duplicate the hydrogen bonding potential of the 3-pyridyl moiety were evaluated. Results demonstrated that basicity, lipophilicity, and linker length were all important for activity (Table 1). Compounds (**17**, **18**, and **19**) with an ethyl linked piperidine or pyrrolidine retained similar activities to the corresponding 3-pyridyl derivatives. The replacement of the cyclic amine for a dimethylamine (**13**) and the introduction of longer linker lengths (**14**) led to drop in potency. Compounds **18** and **19**⁵ showed enhanced hepatic microsomal stability and improved solubility. Furthermore, ligand-lipophilicity efficiency (LLE or LiPE)¹¹ improved for compound **19** (LLE = 3.2) compared with the initial hit **1** (LLE = 2.6). Subsequent analogues were optimized using the ethyl linked pyrrolidine substituent on the amide at R², which displayed the best profile in terms of lipophilicity, activity, and hepatic microsomal stability.

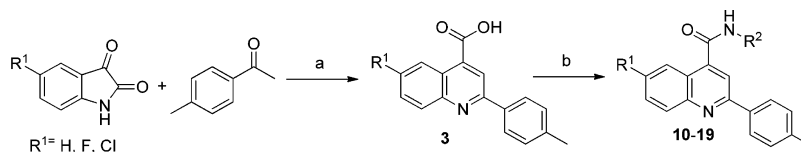
After initial optimization of the R¹ and R² groups, we then turned our attention to the R³ substituent with the aim of improving potency while maintaining lipophilicity at a moderate level (clogP < 3.5). An efficient synthetic route (Scheme 2) was designed for rapid access to a variety of R³ analogues which involved reaction of 5-fluoroisatin or 5-chloroisatin with malonic acid in refluxing acetic acid to provide intermediate **4**.¹² A one pot chlorination and amide formation was achieved by treating 2-hydroxyquinoline-4-carboxylic acid **4** with thionyl chloride in the presence of DMF followed by reaction of the intermediate acid chloride with 2-pyrrolidin-1-ylethanamine in THF at room temperature. Intermediate **5** underwent aromatic nucleophilic substitution with a range of amines under microwave irradiation in acetonitrile to afford compounds **21–30** (Table 2). This route was also used for the synthesis of derivatives with aromatic R³ substituents where a Suzuki coupling of intermediate **5** with the appropriate boronic acid or ester led to compounds **36–39**.

In general, the replacement of the tolyl substituent by an array of primary and secondary amines drove lipophilicity down and also led to improved solubility and hepatic microsomal stability (Table 2). In terms of potency, small heterocycles like 4-amino-3-methyloxazole **20** and *N*-methylpiperazine **21** at the R³ position were not tolerated. However, introduction of the 4-morpholinopiperidine **24** (EC₅₀ = 0.15 μM, LLE = 4.2) moiety

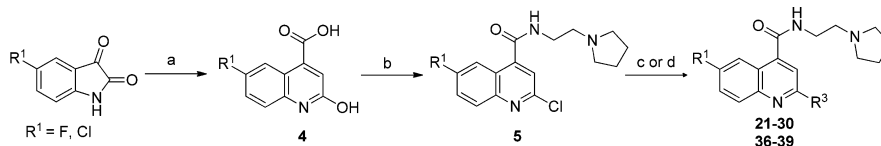
Table 1. Optimization the R¹ and R² Moieties


Comp.	R ¹	R ²	clogP	<i>Pf</i> (3D7) EC ₅₀ (μM)	MRC-5 EC ₅₀ (μM)	MLM ^a Cli (mL/min/g)	Sol (μM) ^b
1^b	Br	3-pyridyl	4.3	0.12	21	5.3	39
10	Cl	3-pyridyl	4.1	0.29	21	-	-
11^b	F	3-pyridyl	3.7	0.35	50	8.6	36
12	H	3-pyridyl	3.6	0.99	>50	-	-
13	Cl		4.2	1.2	9	-	-
14	Cl		4.0	6.7	6	1.1	262
15	Cl		3.3	3.6	26	11	12
16	Cl		3.4	3.3	8	-	-
17	Cl		4.7	0.32	7	-	-
18	Cl		4.1	0.44	7	2.1	13
19^b	F		3.7	0.70	9	3.4	180

^aMLM: mouse liver microsomes. ^bSol: kinetic aqueous solubility. Data for compounds **1**, **11**, and **19** reported previously.⁵

Scheme 1^a

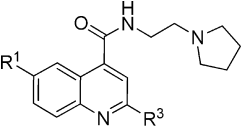
^aConditions: (a) KOH, EtOH/water, 125 °C, microwave, 20 min, 29–58% yield; (b) amine, EDC, HOBT, DMF, room temperature, 16 h, 22–43% yield.

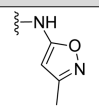










Scheme 2^a

^aConditions: (a) malonic acid, acetic acid, reflux, 16 h, 54%; (b) SOCl₂, DMF, DCM, reflux, 3 h and then 2-pyrrolidin-1-ylethanamine, THF, room temperature, 16 h, 27–43% yield; (c) amine, acetonitrile, 170 °C, microwave, 1 h, 7–54% yield; (d) boronic acid or ester, potassium phosphate, Pd(PPh₃)₄, DMF/water 3/1, 130 °C, microwave, 30 min, 19–73%.

improved both potency and ligand efficiency compared with previous lead compound **18**. Compound **24** displayed good aqueous solubility and moderate mouse hepatic microsomal clearance, which is probably related to the reduction in lipophilicity (clogP = 2.9).

To improve the potency of compound **24**, we investigated other aliphatic amines. The introduction of flexibility at R³ with an aminopropyl morpholine substituent (**25**) led to a further improvement in potency against *P. falciparum* (EC₅₀ = 70 nM) and lipophilic ligand efficiency (LLE = 5.4), with excellent

Table 2. SAR Study of the R³ Substituent: Amines


Comp	R ¹	R ³	clogP	Pf (3D7) EC ₅₀ (μM)	MRC-5 EC ₅₀ (μM)	MLM ^a Cli (mL/min/g)	Sol (μM) ^b
20	F		2.4	>50	>50	-	-
21	F		2.0	34	20	-	-
22	F		2.2	5.8	>50	-	-
23	F		2.6	0.68	25	0.5	220
24	Cl		2.9	0.15	13	2.3	210
25	F		2.1	0.07	41	0.8	230
26	F		1.8	0.19	50	< 0.5	
27	Cl		2.7	0.004	13	2.0	220
28	F		2.9	8.9	19	-	-
29	F		2.8	0.002	>50		
30	Cl		3.1	0.006	31	2.0	230

^aMLM: mouse liver microsomes. ^bKinetic aqueous solubility.

selectivity against mammalian cells. Compound **25** had good aqueous solubility and in vitro hepatic microsomal stability across a range of species (Cli (mL min⁻¹ g⁻¹): mouse 0.8; rat <0.5; human <0.5) and low plasma protein binding (59%). The good in vitro DMPK properties of compound **25** translated into reasonable in vivo pharmacokinetics in mouse (Table 7). Furthermore, compound **25** afforded oral in vivo activity (Table 8) in the *P. berghei* mouse model, with a 93% reduction of parasitemia when dosed orally at 30 mg/kg once a day for four consecutive days. An in vivo pharmacokinetic study in mice for compound **25** showed low clearance, with a moderate volume of distribution and a resultant good half-life. However, oral bioavailability was poor (*F* = 15%). The low systemic exposure of compound **25** was not attributed to high first-pass metabolism due to the low in vitro clearance in mouse microsomes and low in vivo blood clearance but was probably due to poor permeability as highlighted by results in a PAMPA assay (Table 6). Preliminary safety profiling of compound **25** showed a weak affinity to the hERG ion channel (16% inhibition at 11 μM) and an oral maximum tolerated dose (MTD) greater than 300 mg/kg b.i.d. for 4 days. With an attractive overall profile, compound **25** was identified as a key

molecule to declare early lead status for this series, according to the MMV compound development criteria.⁷

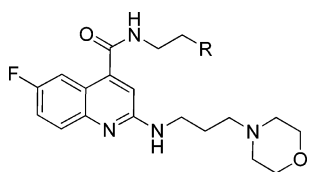
Moving into lead optimization, our focus was to improve potency, permeability, and bioavailability through structural modifications while retaining good physicochemical properties. Reducing the flexibility of compound **25** by shortening the linker length of the aminoalkylmorpholine moiety at R³ was tolerated (**26**). More promising was the 17-fold improvement (EC₅₀ = 4 nM) on antiplasmodial activity obtained when the linker was extended from three to four carbons (**27**). Compound **27** displayed excellent lipophilic ligand efficiency (LLE = 6.2). This improvement on in vitro potency led to enhanced in vivo efficacy (Table 8) with an ED₉₀ of 2.6 mg/kg. In addition with compound **27**, one out of three mice went to cure at 4 × 30 mg/kg (q.d. po). Mouse in vivo pharmacokinetics showed a longer half-life than the early lead **25** as a result of lower in vivo clearance and a slightly higher volume of distribution (Table 7). Despite having improved in vivo potency and half-life, oral bioavailability was still poor, presumably still due to poor permeability (PAMPA *P_e* = 2 nm/s).

Modifications of our lead compound (**25**) focused on modulation of basicity, with the aim of improving permeability. Specifically, we envisaged that lowering basicity would reduce protonation at physiological pH, increase passive permeability, and ultimately improve bioavailability. Early lead **25** has two basic groups, a pyrrolidine (R^2) and a morpholine (R^3) with calculated pK_a values of 8.5 and 7.0 respectively. We first investigated the effect that modifications on the morpholine group at R^3 had on in vitro activity and permeability. We found that the morpholine oxygen was crucial for antiparasitic activity and replacement of the morpholino group by piperidine (**28**) was not tolerated. In contrast, the morpholine nitrogen was not essential for potency and removal, as exemplified by compounds **29** and **30**, was well tolerated leading to single digit nanomolar potency against *P. falciparum*. Moreover, the removal of the basic group at R^3 had not only improved activity but increased permeability more than 20-fold (**30**, $P_e = 48$ nm/s). Furthermore, improved in vitro permeability also translated in vivo, with an increase in oral bioavailability in mice ($F = 23\%$). Despite its shorter half-life, compound **30** was efficacious in the *P. berghei* mouse model with an ED_{90} of 1 mg/kg and achieved a level of in vivo efficacy that met the MMV late lead criteria. However, compound **30** showed very poor rat in vivo exposure that was explained by high intrinsic clearance in rat hepatocytes ($Cl_i = 3 \text{ mL min}^{-1} \text{ g}^{-1}$) which was subsequently confirmed by high hepatic extraction (76%) in a rat hepatic portal vein study. As for other compounds in this series, in vitro hepatic microsomal clearance was consistently low across species (Table 6).

After establishing a link between basicity, PAMPA permeability, and oral bioavailability for the series, we focused on modulating the pK_a of the pyrrolidine group, the stronger of the two basic groups on early lead **25**. Taking into account previous SAR showing that basicity and linker length were important for activity, we designed a short array of analogues with decreasing basicity.¹³ Predicted pK_a ranged from 5.0 for 3-difluoropyrrolidine **35** to 7.9 for 4-fluoropiperidine **31** compared with a predicted pK_a of 8.5 for the lead pyrrolidine **25** (Table 3). As expected, a reduction of basicity resulted in up to 50-fold improvement in permeability. However, potency was dramatically reduced, highlighting the importance of the basic pyrrolidine nitrogen at the R^2 position.

Our attention then turned back to the R^3 position, with the aim of further improving permeability and bioavailability across species. Previous SAR had shown that a methyl group at the para position of an aromatic ring at C-2 was tolerated, so we therefore expanded the range of substituents to include larger groups while maintaining moderate lipophilicity. This strategy also had the advantage of reducing the number of H-bond donors and molecular flexibility, two key factors that can modulate permeability. Early examples of substitution at R^3 showed that introduction of amines, such as dimethylamine (**36**) and morpholine (**37**), reduced lipophilicity and was tolerated in terms of potency compared to compounds **11** and **19** (Table 4). Taking into account the leap in potency observed for **25** with a morpholine attached through a flexible linker at C-2, we prepared compound **2** with a carbon spacer between the phenyl ring and the morpholine to allow improved rotation. The introduction of the benzyl morpholine at R^3 (compound **2**) resulted in a 70-fold increase in potency against *Pf* (3D7) ($EC_{50} = 1$ nM) while retaining good ligand efficiency (LLE = 5.9), more than 30 fold increase in permeability (PAMPA $P_e = 73$ nm/s) and excellent bioavailability in mice ($F = 74\%$).

Table 3. Modulating Basicity of the Amide Substituent



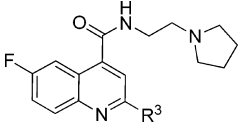
Comp	R	clogP	R group pK_a^a	PAMPA P_e (nm/s) ^b	<i>Pf</i> (3D7) EC_{50} (μ M)
25		2.1	8.5	2.3	0.07
31		2.3	7.9	58	0.9
32		1.4	7.0	24	2.5
33		2.5	7.5	94	15
34		2.5	5.1	120	36
35		2.2	5.0	89	18

^aCalculated pK_a using ChemAxon software. ^bControls: atenolol, 0.7 nm/s; propranolol, 159 nm/s.

Furthermore, in vivo efficacy studies with compound **2** demonstrated complete cure at 4×30 mg/kg po q.d. in a *P. berghei* mouse model, with an ED_{90} of 0.1–0.3 mg/kg (Table 8).

We developed short, four-step synthetic routes for synthesis of **2** and **42** which did not involve the use of palladium catalysis (Scheme 3). Two approaches were employed to synthesize the methyl ketone **8** depending on the availability of commercial starting materials. In the first route, nucleophilic displacement of commercially available 4-(bromomethyl)benzonitrile with morpholine using trimethylamine as a base in DCM gave intermediate **6** which was then converted into the desired methyl ketone **8** by reaction with methylmagnesium bromide followed by an acidic quench. In the second route, radical bromination of commercially available 1-(2-chloro-4-methylphenyl)ethanone with NBS using catalytic amounts of benzoyl peroxide in dichlorobenzene afforded compound **7**, which was subsequently reacted with morpholine to yield the methyl ketone **8**. As described earlier, a Pfitzinger reaction of 5-fluoroisatin with the appropriate methyl ketone led to acid **9**. The final amides were prepared using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) as coupling agent and *N*-methylmorpholine in DCM at room temperature. Details of other synthetic routes for individual compounds are described in the Supporting Information.

As highlighted above, a basic group is not required for activity at R^3 . Thus, derivatives of **2** with either reduced basicity (**40**, **41**, and **42**) or a nonbasic substituent (**38**, **43**, **44**) at R^3 showed excellent potency with the exception of amide **39**. The introduction of a conformationally restricted bridge amide as exemplified by compound **39** is detrimental for potency, possibly because it does not allow rotation of the morpholine group to adopt the optimal orientation for binding. The importance of the orientation of the morpholine substituent for activity is also highlighted by compound **45**. In this case, a change of the methylmorpholine group from para to the meta position led to a weakly active compound.

Table 4. SAR Study of the R³ Substituent: Aromatic Groups


Comp	R ³	MW	clogP	R ³ group pK _a ^a	<i>Pf</i> (3D7) EC ₅₀ (μM)	MRC-5 EC ₅₀ (μM)
36		406	3.3	4.3	0.81	10
37		448	3.3	0.4	0.60	17
2 ^b		463	3.3	7.2 (6.8)	0.001	24
38		513	2.6	Non basic	0.004	21
39		476	2.5	Non basic	0.14	>50
40		480	3.4	6.1(6.1)	0.0006	19
41		480	3.4	6.4 (6.3)	0.002	41
42		497	3.8	6.1(6.3)	0.003	>50
43		511	2.4	3.4	0.0006	41
44		460	3.2	Non basic	0.007	-
45		463	3.3	6.9	11	-

^aCalculated pK_a using ChemAxon software. Experimental pK_a using potentiometric titration is shown in parentheses. ^bData for this compound reported previously.⁵

On the basis of their excellent potency, good permeability, microsomal stability, and solubility (Table 6), compounds **2**, **40**, **41**, **43**, and **44** were progressed for efficacy studies in mice dosing at 1 mg/kg for 4 days (Table 8). Compounds **40**, **43**, and **44** showed excellent activity at this low dose, with reductions of parasitemia above 99%. Compound **41** showed the best survival time (14 days) comparable with **2**.

Once an optimal R³ substituent had been identified, we turned our attention back to the R² substituent to see if it was possible to improve the profile of compound **2** (Table 5). As highlighted before, lowering basicity at R² results in a reduction in potency. The replacement of the pyrrolidine for a morpholine in compound **46** led to a 12-fold drop in potency. The amide NH is also important for activity, as capping with a methyl group resulted in an 87-fold decrease in potency against

P. falciparum (3D7) (**47**, EC₅₀ = 87 nM). Finally, it is possible to reduce the size of the ring on the R² substituent and retain activity as shown by compounds **48** and **49**. Compound **49** showed excellent in vivo activity in the *P. berghei* mouse model at 4 × 10 mg/kg and 4 × 3 mg/kg. (Table 8). However, none of these compounds offered a particular advantage to compound **2**.

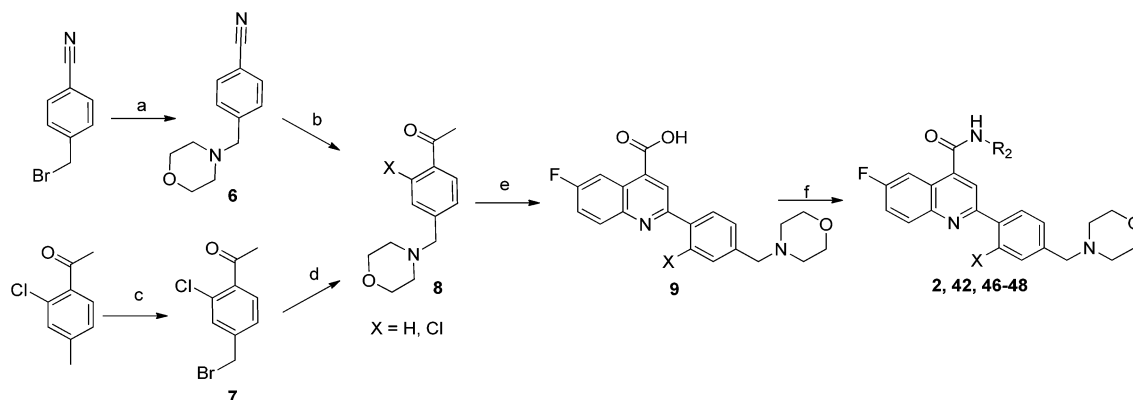
Although in vitro DMPK data and in vivo efficacy in the *P. berghei* model were comparable for compound **2** and the fluorinated derivative **41**, oral bioavailability in rat (33% for **41** vs 84% for **2**) was lower and rat intravenous elimination half-life (4 h for **41** vs 10 h for **2**) was shorter for **41** (Table 9). Therefore, compound **2** showed the best overall profile for further progression from this novel quinoline-4-carboxamide series (Table 9 and Figure 2). Compound **2** fulfilled the efficacy and DMPK requirements for a late lead according to the MMV criteria and, following further profiling, was selected as a preclinical candidate by MMV. The studies required to profile **2** for candidate selection have been described elsewhere.⁵

We also profiled key compounds of this series for their activity against different life stages of the malaria parasite life cycle (Table 10). Following a mosquito bite (blood meal), sporozoites are injected into the skin and migrate in the bloodstream to the liver, where they invade liver hepatocytes and then develop into liver schizonts. Compounds active against liver schizonts can potentially prevent disease development and be used in chemoprotection. Compounds **2**, **27**, **30**, and **38** showed low nanomolar activity against the live schizont forms of *Plasmodium yoelli*.¹⁴ Several compounds were also tested in vitro against *P. falciparum* late stage (IV–V) gametocytes. Stage V gametocytes, typically insensitive to antimalarial drugs, are infectious to mosquitoes and responsible for the transmission of the disease. 4-Quinolincarboxamides, in particular compounds **2** and **30**, are potent antigametocytocidal (stage IV–V) with nanomolar activities.¹⁵ The ability of compounds of this series to block transmission was further tested in the *P. berghei* ookinete development assay, which simulates in vitro the first 24 h of parasite development in the mosquito midgut, from mature gametocyte transformation into gametes, through fertilization and to mature ookinete development. Compounds **2**, **27**, **30** and **38** showed nanomolar potency in this assay.^{14b}

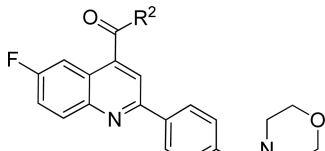
Finally, compounds **2** and **30** were tested against several *Plasmodium falciparum* drug resistant strains (K1, W2, 7G8, TM90C2A, D6, and V1/S) showing similar levels of activity across strains^{14b} (Table 11).

CONCLUSION

We have evolved a malaria phenotypic hit series, which started with moderate in vitro activity and selectivity but suboptimal metabolic stability and physicochemical properties, into an early lead compound **25** with improved potency, ligand efficiency, metabolic stability, and in vivo efficacy. The lead optimization phase focused on improving low oral bioavailability caused by the poor permeability of the early lead. The most advanced quinoline-4-carboxamides showed exceptional in vitro and in vivo activities, with a reduction of parasitemia of more than 99% when administered at low doses (4 × 1 mg/kg, 4 days, po) in the *P. berghei* mouse model. In addition to potent intraerythrocyte activity, compounds in this series showed similar potency against liver schizonts, gametocytes, and ookinetes in vitro. Furthermore, a combination of in vitro and in vivo activities across the different stages of the malaria

Scheme 3^a

^aConditions: (a) morpholine, Et₃N, DCM, 16 h, 72% yield; (b) MeMgBr, toluene, reflux, 4 h and then a 10% aqueous HCl, reflux, 1 h, 70% yield; (c) NBS, benzoyl peroxide, dichlorobenzene, 140 °C, 16 h, 70% yield; (d) morpholine, K₂CO₃, acetonitrile, 40 °C, 16 h, 64% yield; (e) 5-fluoroisatin, KOH, EtOH, 120 °C, microwave, 20 min, 30–76% yield; (f) amine, CDMT, N-methylmorpholine, DCM, 20–61% yield.

Table 5. SAR Study of the R² Substituent


Comp	R ²	MW	clogP	R ² group pK _a ^a	Pf (3D7) EC ₅₀ (μM)	HepG2 EC ₅₀ (μM)
46		479	2.7	6.3	0.012	-
47		477	3.3	8.6	0.087	>50
48		448	3.0	9.3	0.002	>50
49		448	3.0	7.8	0.004	41

^aCalculated pK_a using ChemAxon software.

parasite life cycle demonstrated the potential of this novel quinoline-4-carboxamide series to meet a number of the MMV's malaria target candidate profiles (TCPs), as previously described.⁷

Compound **2** has been extensively profiled⁵ in vitro and in vivo and displays activity against multiple life-cycle stages of the parasite with a long half-life in preclinical animal studies. With

this profile, compound **2** has the potential for single dose treatment of malaria as part of a combination therapy, together with transmission blocking potential (TCP2 and TCP3b).⁷ It also has the potential for chemoprotection (TCP4).⁷ Compound **2** was active against parasites that show resistance to currently used antimalarials and has a novel mode of action through inhibition of elongation factor 2 (*PfeEF2*), which is involved in protein synthesis. The favorable potency, selectivity, DMPK properties, efficacy, safety profile, and novel mechanism of action support the progression of **2** toward clinical development.

EXPERIMENTAL SECTION

Chemistry. General. Solvents and reagents were purchased from commercial suppliers and used without further purification. Dry solvents were purchased in Sure/Seal bottles stored over molecular sieves. Reactions using microwave irradiation were carried out in a Biotage Initiator microwave. Normal phase TLCs were carried out on precoated silica plates (Kieselgel 60 F₂₅₄, BDH) with visualization via UV light (UV 254/365 nm) and/or ninhydrin solution. Flash chromatography was performed using Combiflash Companion Rf (Teledyne ISCO) and prepacked silica gel columns purchased from Grace Davison Discovery Science or SiliCycle. Mass-directed preparative HPLC separations were performed using a Waters HPLC (2545 binary gradient pumps, 515 HPLC make-up pump, 2767 sample manager) connected to a Waters 2998 photodiode array and a Waters 3100 mass detector. Preparative HPLC separations were performed with a Gilson HPLC (321 pumps, 819 injection module, 215 liquid handler/injector) connected to a Gilson 155 UV/vis detector. On both instruments, HPLC chromatographic separations were conducted using Waters XBridge C18 columns, 19 mm × 100

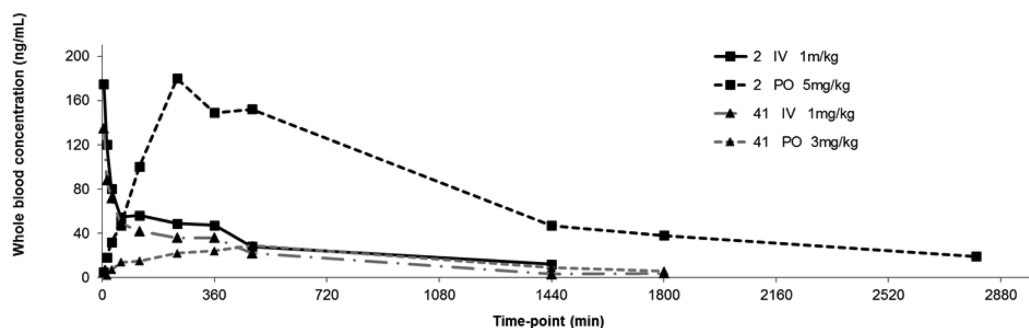


Figure 2. Mean blood concentration time profile of compounds **2** and **41** following intravenous or oral administration to male Sprague Dawley rats.

Table 6. Key in vitro DMPK Data for Selected Analogues

compd	PAMPA P_e (nm/s) ^a	Sol. (μ M)	MLM ^b Cl _i (mL min ⁻¹ g ⁻¹)	RLM ^c Cl _i (mL min ⁻¹ g ⁻¹)	HLM ^d Cl _i (mL min ⁻¹ g ⁻¹)	PPB (%) ^e	hERG ^f IC ₅₀ (μ M)
25	2.3	232	0.8		<0.5	59	>11
27	2	217	2.0		<1	49	>11
30	48	232	2	0.8	1	77	>11
2 ^g	73	216	<0.5	≤0.8	≤1.1	63	16
38	29	109	1.9			75	11
40	63	>208	1.0				
41	132	208	<0.5	<0.5	1.5		
42		>201	0.5			56	
43	14	171	<0.5	0.8			
44	49	>217	2.0	0.6	2		
46		>210	<0.5				
47	137	>210	<0.5				
48	49		2				
49	75	71	0.5				

^aControls: atenolol, 0.2–4.6 nm/s; propranolol, 103–159 nm/s. ^bMLM: mouse liver microsome. ^cRLM: rat liver microsome. ^dHLM: human liver microsome. ^eMouse plasma protein binding. ^fMeasured using IonWorks Patch Clamp electrophysiology. ^gData for this compound reported previously.⁵

Table 7. In vivo Pharmacokinetic Profile in Mice of Key Compounds

compd	intravenous at 3 mg/kg			oral at 10 mg/kg			
	Cl _b (mL min ⁻¹ kg ⁻¹)	Vd _{ss} (L/kg)	T _{1/2} (h)	C _{max} (ng/mL)	AUC (ng·min/mL)	T _{max} (h)	F (%)
25	14	3	3.2	315	92922	2	15
27	4	3.5	12.5	579	176115	2	16
30	34	7.4	2.9	193	72728	0.5	23
2 ^a	12 ^b	15 ^b	16 ^b	90 ^c	179272 ^c	1 ^c	74

^aData for this compound reported previously.⁵ ^biv dose: 1 mg/kg. ^cpo dose: 3 mg/kg.

Table 8. In vivo Oral Activity in the *P. berghei* Mouse Model Peter's Test

compd	4 × 30 mg/kg			4 × 10 mg/kg			4 × 3 mg/kg			4 × 1 mg/kg		
	activity (%)	survival (days)	cure	activity (%)	survival (days)	cure	activity (%)	survival (days)	cure	activity (%)	survival (days)	cure
25	93.0	7										
27	99.8	22	1/3	99.7	15		96.0	9		48.0	6.0	
30	99.9	10		99.9	8		98.0	6		90.0	7	
2	99.9	>30	3/3	99.9	>30	3/3	99.9	25	2/3	99.9	14	
40										99.8	11.0	
41										99.9	14	
43										99.0	7	
44										<40		
49				99.1	20.3	1/3	99.2	9.3				

Table 9. In vivo Pharmacokinetic Parameters in Male Sprague Dawley Rat

compd	intravenous at 1 mg/kg			oral at 3 mg/kg			
	Cl _b (mL min ⁻¹ kg ⁻¹)	Vd _{ss} (L/kg)	T _{1/2} (h)	C _{max} (ng/mL)	T _{max} (h)	AUC (ng·min/mL)	F (%)
2 ^a	18	15	10	180 ^b	4 ^b	200542 ^b	84
41	32	10	4	29	8	30400	33

^aData for this compound reported previously.⁵ ^bpo dose: 5 mg/kg.

mm, 5 μ m particle size, using 0.1% ammonia in water (solvent A) and acetonitrile (solvent B) as mobile phase. ¹H NMR, ¹⁹F NMR spectra were recorded on a Bruker Avance DPX 500 spectrometer (¹H at 500.1 MHz, ¹⁹F at 470.5 MHz) or a Bruker Avance DPX 300 (¹H at 300 MHz). Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broadened (br), or a combination thereof. Coupling constants (*J*) are quoted to the nearest 0.1 Hz. Low resolution electrospray (ES) mass spectra were recorded on a Bruker Daltonics MicrOTOF mass spectrometer run in positive mode. High

resolution mass spectroscopy (HRMS) was performed using a Bruker Daltonics MicroToF mass spectrometer. LC–MS analysis and chromatographic separation were conducted with a Bruker Daltonics MicrOTOF mass spectrometer or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, where both instruments were connected to an Agilent diode array detector. The column used was a Waters XBridge column (50 mm × 2.1 mm, 3.5 μ m particle size), and the compounds were eluted with a gradient of 5–95% acetonitrile/water + 0.1% ammonia. All final compounds showed chemical purity of ≥95% as determined by the

Table 10. Activity against *Plasmodium* Life Cycle Stages

compd	<i>Pf</i> (3D7) EC ₅₀ (nM)	<i>Py</i> liver stage EC ₅₀ (nM)	<i>Pf</i> GAM IV-V EC ₅₀ (nM) ^a	<i>Pb</i> pokinete EC ₅₀ (nM)
27	4	18	104	5
30	6	1	39	14
2	1	1 ^b	24	5 ^b
38	4	4	152	15

^aRun in duplicate. Reference controls: pyronaridine EC₅₀ = 3108 nM, tafenoquine EC₅₀ = 5250 nM, artemisinin EC₅₀ = 0.8 nM. ^bData reported previously.⁵

Table 11. Activity against *Plasmodium falciparum* Resistant Strains^a

compd	EC ₅₀ (nM)						
	NF5	K1	W2	7G8	TM90C2A	D6	V1/S
30	0.5	0.8	0.6	0.7	0.5	0.7	0.9
2	0.3	0.4	0.4	0.4	0.4	0.4	0.7

^aData for compound 2 have been previously reported.⁵

UV chromatogram (190–450 nm) obtained by LC–MS analysis. Unless otherwise stated herein reactions have not been optimized.

General Procedure A: Preparation of 2-(*p*-Tolyl)quinolone-4-carboxylic Acids (3). To a mixture of the corresponding isatin (5 mmol) in ethanol (10 mL) were added the corresponding acetophenone (5 mmol), water (10 mL), and potassium hydroxide (2.80 g, 50 mmol). The reaction mixture was heated under microwave irradiation at 125 °C for 20 min. The resulting dark red colored solution was diluted with water (50 mL) and acidified by adding HCl (2 M, 30 mL). The resulting precipitate (yellow to ochre in color) was collected by filtration, washed with water (50 mL), ethyl acetate (100 mL), and dichloromethane (25 mL). The remaining solid was used in the next step without further purification.

6-Chloro-2-(*p*-tolyl)quinolone-4-carboxylic Acids (3, R¹ = Cl). Prepared using general procedure A. Yield, 58% (861 mg); ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.82 (d, *J* = 2.3 Hz, 1H), 8.44 (s, 1H), 8.19 (d, *J* = 8.2 Hz, 2H), 8.13 (d, *J* = 9.0 Hz, 1H), 7.82 (dd, *J* = 2.3, 9.0 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 2.41 (s, 3H) ppm; LC–MS *m/z* 298 (M + H)⁺.

2-(*p*-Tolyl)quinolone-4-carboxylic Acids (3, R¹ = H). Prepared using general procedure A. Yield, 29% (384 mg); ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.65 (d, 1H, *J* = 8.5 Hz), 8.45 (s, 1H), 8.22 (d, 2H, *J* = 8.2 Hz), 8.16 (d, 1H, *J* = 7.8 Hz), 7.85 (ddd, 1H, *J* = 1.4, 6.9, 8.4 Hz), 7.70 (ddd, 1H, *J* = 1.3, 6.9, 8.3 Hz), 7.39 (d, 2H, *J* = 7.9 Hz), 2.41 (s, 3H) ppm; LC–MS *m/z* 251 (M + H)⁺.

General Procedure B: Preparation of 2-(*p*-Tolyl)quinolone-4-carboxamides (10–18). To a solution of quinolinecarboxylic acid 3 (0.5 mmol) in DMF (3 mL) was added diisopropylethylamine (0.07 mL, 0.75 mmol) followed by EDC (144 mg, 0.75 mmol) and HOBt (101 mg, 0.75 mmol). After 5 min the corresponding amine (1 mmol) was added followed by DMF (2 mL). The reaction mixture was stirred at room temperature overnight. The reaction was diluted with water (50 mL) and brine (10 mL) and extracted with ethyl acetate (30 mL). Solvents were removed, and the resulting off white solid was washed with dichloromethane and filtered. Amides that were soluble in dichloromethane were purified by column chromatography on silica.

6-Chloro-*N*-(pyridine-3-yl)-2-(*p*-tolyl)quinoline-4-carboxamide (10). Prepared using general procedure B. Yield, 25% (47 mg); ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.06 (br s, 1H), 8.96 (d, *J* = 2.0 Hz, 1H), 8.48 (s, 1H), 8.39 (dd, *J* = 1.4, 4.8 Hz, 1H), 8.30–8.25 (m, 4H), 8.18 (d, *J* = 9.0 Hz, 1H), 7.87 (d, *J* = 2.4, 9.0 Hz, 1H), 7.47 (dd, *J* = 4.8, 8.3 Hz, 1H), 7.41 (d, *J* = 8.3 Hz, 2H), 2.41 (s, 3H) ppm; HRMS for C₂₂H₁₇ClN₃O (M + H)⁺ calcd 374.1055, found 374.1051.

***N*-(Pyridine-3-yl)-2-(*p*-tolyl)quinoline-4-carboxamide (12).** Prepared using general procedure B. Yield, 41% (69 mg); ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.10 (s, 1H), 9.02 (d, *J* = 1.9 Hz, 1H), 8.44–8.43 (m, 2H), 8.34–8.31 (m, 3H), 7.92–7.89 (m, 1H), 7.73–

7.70 (m, 1H), 7.52 (dd, *J* = 4.8, 8.2 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 2.46 (s, 3H) ppm; LC–MS *m/z* 340 (M + H)⁺.

6-Chloro-*N*-(3-(dimethylamino)ethyl)-2-(*p*-tolyl)quinoline-4-carboxamide (13). Prepared using general procedure B. Product was purified by column chromatography using a 4 g silica gel cartridge. Solvent A: DCM. Solvent B: 20% MeOH in DCM. Gradient: 3 min hold 100% A, 15 min ramp to 50% B, 2 min hold at 50% B. Fractions containing product were combined and concentrated to dryness under reduced pressure to obtain 13. Yield, 27% (50 mg); ¹H NMR (500 MHz, CDCl₃) δ 8.20 (d, *J* = 2.4 Hz, 1H), 8.08–8.05 (m, 1H), 7.99 (d, *J* = 8.2 Hz, 2H), 7.84 (s, 1H), 7.64 (dd, *J* = 2.2, 9.0 Hz, 1H), 7.33–7.28 (2H, m), 7.06 (1H, s), 3.65 (q, *J* = 5.6 Hz, 2H), 2.62 (t, *J* = 5.9 Hz, 2H), 2.45 (s, 3H), 2.33 (s, 6H); LC–MS *m/z* 368 (M + H)⁺.

6-Chloro-*N*-(3-(dimethylamino)propyl)-2-(*p*-tolyl)quinoline-4-carboxamide (14). Prepared using general procedure B. Yield, 28% (53 mg); ¹H NMR (500 MHz, CDCl₃) δ 8.51 (br s, 1H), 8.34 (d, 1H, *J* = 2.4 Hz), 8.08 (d, 1H, *J* = 9.0 Hz), 8.04 (d, 2H, *J* = 8.2 Hz), 7.92 (s, 1H), 7.65 (dd, 1H, *J* = 2.1, 9.0 Hz), 7.30 (d, 2H, *J* = 8.2 Hz), 3.66 (q, 2H, *J* = 5.6 Hz), 2.51 (t, 2H, *J* = 5.6 Hz), 2.42 (s, 3H), 2.19 (s, 6H), 1.84–1.79 (m, 2H) ppm; HRMS for C₂₂H₂₅ClN₃O (M + H)⁺ calcd 382.1681, found 382.1673.

6-Chloro-*N*-(2-morpholinoethyl)-2-(*p*-tolyl)quinoline-4-carboxamide (15). Prepared using general procedure B. Yield, 22% (45 mg); ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.88 (t, *J* = 5.6 Hz, 1H), 8.31 (d, *J* = 2.4 Hz, 1H), 8.24–8.22 (m, 2H), 8.23 (s, 1H), 8.19 (s, 1H), 8.14 (d, *J* = 9.0 Hz, 1H), 7.85 (dd, *J* = 2.4, 9.0 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 2H), 3.65 (t, *J* = 4.5 Hz, 4H), 3.52 (q, *J* = 6.4 Hz, 2H), 2.57–2.54 (m, 2H), 2.41 (s, 3H) ppm; LC–MS *m/z* 410 (M + H)⁺.

6-Chloro-*N*-(2-(4-methylpiperazin-1-yl)ethyl)-2-(*p*-tolyl)quinoline-4-carboxamide (16). Prepared using general procedure B. Yield, 27% (55 mg); ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.30 (d, 1H, *J* = 2.4 Hz), 8.23 (d, *J* = 8.2 Hz, 2H), 8.18–8.13 (m, 2H), 7.85 (dd, *J* = 2.4, 9.0 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 2H), 3.54–3.48 (m, 2H), 2.55 (t, *J* = 6.4 Hz, 4H), 2.52–2.50 (m, 4H), 2.41 (s, 5H), 2.22 (s, 3H) ppm; LC–MS *m/z* 408 (M + H)⁺.

6-Chloro-*N*-(2-(piperidin-1-yl)ethyl)-2-(*p*-tolyl)quinoline-4-carboxamide (17). Prepared using general procedure B. Yield, 27% (55 mg); ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.93 (t, *J* = 5.6 Hz, 1H), 8.39 (d, *J* = 2.4 Hz, 1H), 8.33–8.31 (m, 2H), 8.27 (s, 1H), 8.23 (d, *J* = 9.0 Hz, 1H), 7.94 (dd, *J* = 2.4, 9.0 Hz, 1H), 7.49 (d, *J* = 8.1 Hz, 2H), 3.60 (q, *J* = 6.4 Hz, 2H), 2.56–2.49 (m, 7H), 1.67–1.63 (m, 4H), 1.53–1.49 (m, 2H) ppm; LC–MS *m/z* 408 (M + H)⁺.

6-Chloro-*N*-(2-(pyrrolidin-1-yl)ethyl)-2-(*p*-tolyl)quinoline-4-carboxamide (18). Prepared using general procedure B. Yield, 43% (85 mg); ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.87 (t, *J* = 5.7 Hz, 1H), 8.30 (d, *J* = 2.7 Hz, 1H), 8.22 (d, *J* = 8.8 Hz, 2H), 8.17 (s, 1H), 8.12 (d, *J* = 10.0 Hz, 1H), 7.82 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.38 (d, *J* = 8.1 Hz, 2H), 3.50 (q, *J* = 5.7 Hz, 2H), 2.65 (t, *J* = 7.8 Hz, 2H), 2.56–2.54 (m, 4H), 2.30 (s, 3H), 1.73 (m, 4H) ppm; HRMS for C₂₃H₂₅ClN₃O (M + H)⁺ calcd 394.1681, found 394.1663.

6-Fluoro-2-hydroxyquinoline-4-carboxylic Acid (4, R¹ = F). A stirred suspension of 5-fluoroisatin (10.00 g, 61 mmol) and malonic acid (18.91 g, 182 mmol) in acetic acid (400 mL) was refluxed for 16 h. Acetic acid was removed under reduced pressure, and the residue was suspended in water (400 mL), filtered, and washed with water (300 mL) to give a brown solid. The solid was stirred in NaHCO₃ saturated aqueous solution (800 mL), and the insoluble material was filtered off. The filtrate was acidified to pH 1–2 with concentrated HCl, and the resulting precipitate was filtered, washed with water (300 mL), and dried. The resulting pale yellow solid was used directly for synthesis of 4 without further purification. Yield, 54% (10 g); ¹H NMR (500 MHz, DMSO-*d*₆) δ 2.08 (s, 1.5 H), 7.01 (s, 1H), 7.35–7.37 (m, 0.5 H)*, 7.48–7.49 (m, 2H), 7.64–7.68 (m, 0.9H)*, 8.01–8.04 (m, 1H), 12.29 (brs, 1H), 13.38 (brs, 0.7H)* ppm; LC–MS purity 63%, *m/z* 208 (M + H)⁺; * corresponds to impurity.

6-Chloro-2-hydroxyquinoline-4-carboxylic Acid (4, R¹ = Cl). A stirred suspension of 6-chloroisatin (10.00 g, 55 mmol) and malonic acid (17.00 g, 165 mmol) in acetic acid (400 mL) was refluxed for 16 h. Acetic acid was removed under reduced pressure, and the residue was suspended in water (400 mL), filtered, and washed with water

(300 mL) to give a gray solid. The solid was stirred in NaHCO_3 saturated aqueous solution (800 mL), and the insoluble material was filtered off. The filtrate was acidified to pH 1–2 with concentrated HCl, and the precipitate was filtered, washed with water, and dried. The resulting pale yellow solid was used for the synthesis of **4** without further purification. Yield, 54% (9.5 g, 42 mmol); ^1H NMR (500 MHz; $\text{DMSO}-d_6$) δ 7.00 (s, 1H), 7.42 (d, 1H, J = 8.8 Hz), 7.58 (d, 0.3 H, J = 8.9 Hz)*, 7.60–7.62 (m, 1.3 H), 7.81 (dd, 0.3H, J = 2.3 Hz, J = 8.9 Hz)*, 8.29 (d, 1H, J = 2.4 Hz), 12.28 (brs, 1H), 13.22 (brs, 0.3 H)* ppm; LC–MS purity 65%, m/z 224 ($M + H$)*; * corresponds to impurity.

2-Chloro-6-fluoro-*N*-(2-pyrrolidin-1-ylethyl)quinoline-4-carboxamide (5, $R^1 = F$). To a stirred suspension of 6-fluoro-2-hydroxyquinoline-4-carboxylic acid (**4**, $R^1 = F$) (10.00 g, 48 mmol) in anhydrous DCM (350 mL) were added anhydrous DMF (7 mL) and thionyl chloride (14 mL, 193 mmol) under argon at room temperature. The mixture was refluxed for 3 h and then allowed to cool to room temperature. The solvents were removed under reduced pressure, and the residue was dissolved in anhydrous THF (350 mL) under argon. 2-Pyrrolidin-1-ylethanamine (18 mL, 145 mmol) was added, and the reaction was stirred at room temperature for 16 h. Solvents were removed under vacuum and the residue was partitioned between NaHCO_3 saturated aqueous solution (250 mL) and DCM (2 \times 200 mL). The organic layers were combined, dried over MgSO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography using a 120 g silica gel cartridge. Solvent A: DCM. Solvent B: 10% MeOH– NH_3 in DCM. Gradient: 2 min hold 100% A followed by 18 min ramp to 30% B and then 15 min hold at 30% B. Fractions containing product were combined and concentrated to dryness under reduced pressure to obtain the desired compound as an off-white solid. Yield, 27% (4.59 g); ^1H NMR (500 MHz; CDCl_3) δ 8.07 (dd, J = 5.4, 9.2 Hz, 1H), 7.99 (dd, J = 2.8, 9.7 Hz, 1H), 7.56 (ddd, J = 2.8, 7.9, 9.2 Hz, 1H), 7.53 (s, 1H), 6.88 (brs, 1H), 3.65–3.69 (m, 2H), 2.81 (t, J = 5.5 Hz, 2H), 2.64 (brs, 4H), 1.83–1.85 (m, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –110.03 ppm; LC–MS m/z 322 ($M + H$)*.

2,6-Chloro-*N*-(2-pyrrolidin-1-ylethyl)quinoline-4-carboxamide (5, $R^1 = \text{Cl}$). To a stirred suspension of 6-chloro-2-hydroxyquinoline-4-carboxylic acid (**3**) (8.50 g, 38 mmol) in anhydrous DCM (250 mL) were added anhydrous DMF (2 mL) and thionyl chloride (11 mL, 152 mmol) at room temperature under argon. The mixture was refluxed for 3 h and then allowed to cool to room temperature. The solvents were removed under reduced pressure, and the residue was dissolved in anhydrous THF (300 mL) under argon. 2-Pyrrolidin-1-ylethanamine (14 mL, 114 mmol) was added, and the reaction was stirred at room temperature for 16 h. Solvents were removed under vacuum and the residue was partitioned between NaHCO_3 saturated aqueous solution (250 mL) and DCM (2 \times 250 mL). The organic layers were combined, dried over MgSO_4 , filtered, and evaporated under reduced pressure. The crude was purified by column chromatography using a 120 g silica gel cartridge. Solvent A: DCM. Solvent B: 10% MeOH– NH_3 in DCM. Gradient: 2 min hold 100% A followed by 18 min ramp to 30% B and then 15 min hold at 30% B. The relevant fractions were combined and concentrated to dryness under reduced pressure to obtain the desired product as an off-white solid. Yield, 43% (5.6 g); ^1H NMR (500 MHz; CDCl_3) δ 8.27 (d, J = 2.3 Hz, 1H), 7.97 (d, J = 9.0 Hz, 1H), 7.70 (dd, J = 2.3, 9.0 Hz, 1H), 6.83 (brs, 1H), 7.48 (s, 1H), 3.64 (dt, J = 5.2, 11.6 Hz, 2H), 2.74–2.77 (m, 2H), 2.57–2.60 (m, 4H), 1.78–1.81 (m, 4H) ppm; LC–MS m/z 338 ($M + H$)*.

6-Fluoro-2-((3-methylisoxazol-5-yl)amino)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (20). To a solution of **5** ($R^1 = F$) (65 mg, 0.2 mmol) and 5-amino-3-methylisoxazole (39 mg, 0.4 mmol), BINAP (33 mg, 0.05 mmol) in dioxane (4 mL) were added sodium *tert*-butoxide (38 mg, 0.4 mmol) and palladium acetate (9 mg, 0.04 mmol). Reaction was heated at 115 $^\circ\text{C}$ overnight in a sealed tube. Reaction crude was filtered through Celite, and the filtrate was partitioned between water (5 mL) and DCM (25 mL). Organic phase was dried over MgSO_4 , and solvents were removed under reduced pressure. Product was purified by column chromatography on a 4 g

silica cartridge using A (DCM) and B (10% MeOH– NH_3 in DCM). Fractions containing product were pooled together to obtain **20** as a white solid. Yield, 13% (10 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.94 (s, 1H), 7.62–7.57 (m, 2H), 7.32 (dd, J = 2.8, 8.2 Hz, 1H), 6.88 (s, 1H), 5.68–5.63 (m, 1H), 3.80 (d, J = 4.6 Hz, 2H), 2.95 (t, J = 5.3 Hz, 2H), 2.80 (s, 4H), 2.27 (s, 3H), 1.77 (s, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –117.06 ppm; LC–MS m/z 384 ($M + 1$).

General Procedure C: Preparation of 2-Amino-4-carboxamides (21–30). A solution of **5** (1 equiv) and the corresponding amine (3 equiv) in acetonitrile (2.5 mL) in a microwave vial was heated at 170 $^\circ\text{C}$ for 1 h under microwave irradiation. Solvents were removed and product was purified by column chromatography on 4 g silica cartridges using A (DCM) and B (10% MeOH– NH_3 in DCM).

6-Fluoro-2-(4-methylpiperazin-1-yl)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (21). Prepared using general procedure C starting from **5** ($R^1 = F$) (0.2 mmol, 65 mg), light yellow solid. Yield, 26% (20 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.72–7.64 (m, 2H), 7.34–7.29 (m, 1H), 7.13 (s, 1H), 6.83 (s, 1H), 3.76 (dd, J = 5.0, 5.0 Hz, 4H), 3.68–3.62 (m, 2H), 2.78 (dd, J = 5.9, 5.9 Hz, 2H), 2.61–2.54 (m, 8H), 2.38 (s, 3H), 1.81–1.78 (m, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –118.04 ppm; LC–MS m/z 386 ($M + 1$).

2-(5,6-Dihydroimidazo[1,2-*a*]pyrazine-7(8*H*)-yl)-6-fluoro-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (22). Prepared using general procedure C starting from **5** ($R^1 = F$) (0.6 mmol, 96 mg), white solid. Yield, 18% (15 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.78–7.71 (m, 2H), 7.39–7.34 (m, 1H), 7.23 (s, 1H), 7.13–7.11 (m, 1H), 7.04–7.03 (m, 1H), 6.88–6.87 (m, 1H), 4.88 (s, 2H), 4.28 (t, J = 5.4 Hz, 2H), 4.14 (t, J = 5.4 Hz, 2H), 3.67–3.62 (m, 2H), 2.77 (t, J = 6.0 Hz, 2H), 2.60–2.57 (m, 4H), 1.82–1.77 (m, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –117.16 ppm; LC–MS m/z 409 ($M + 1$).

6-Fluoro-2-(4-morpholinopiperidin-1-yl)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (23). Prepared using general procedure C starting from **5** ($R^1 = F$) (0.2 mmol, 65 mg), yellow solid. Yield, 22% (20 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.71–7.64 (m, 2H), 7.35–7.30 (m, 1H), 7.14 (s, 1H), 6.77 (s, 1H), 4.55 (d, J = 13.2 Hz, 2H), 3.74 (t, J = 4.7 Hz, 4H), 3.66–3.62 (m, 2H), 2.98–2.93 (m, 2H), 2.77 (t, J = 6.0 Hz, 2H), 2.61–2.57 (m, 8H), 2.52–2.43 (m, 1H), 1.80 (t, J = 6.6 Hz, 4H), 1.61–1.50 (m, 2H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –118.31 ppm; LC–MS m/z 456 ($M + 1$).

6-Chloro-2-(4-morpholinopiperidin-1-yl)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (24). Prepared using general procedure C starting from **5** ($R^1 = \text{Cl}$) (0.2 mmol, 70 mg), yellow solid. Yield, 31% (28 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.97 (d, J = 2.2 Hz, 1H), 7.65 (d, J = 8.8 Hz, 1H), 7.50 (dd, J = 2.4, 8.8 Hz, 1H), 7.15 (s, 1H), 6.63–6.63 (m, 1H), 4.59 (d, J = 13.2 Hz, 2H), 3.75 (t, J = 4.6 Hz, 4H), 3.66 (q, J = 5.6 Hz, 2H), 3.05–2.98 (m, 2H), 2.78 (dd, J = 5.8, 5.8 Hz, 2H), 2.63–2.58 (m, 8H), 2.53–2.47 (m, 1H), 2.00 (d, J = 12.5 Hz, 2H), 1.82 (dd, J = 3.2, 6.5 Hz, 4H), 1.65–1.53 (m, 2H) ppm; LC–MS m/z 473 ($M + 1$).

6-Fluoro-2-((3-morpholinopropyl)amino)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (25). Prepared using general procedure C starting from **5** ($R^1 = F$) (0.75 mmol, 241 mg), off white solid. Yield, 34% (110 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.52–7.48 (m, 2H), 7.19–7.16 (m, 1H), 6.36–6.35 (m, 1H), 6.02 (s, 1H), 3.64 (br s, 4H), 3.56 (q, J = 5.6 Hz, 2H), 3.22 (br s, 2H), 2.70 (t, J = 5.9 Hz, 2H), 2.53 (br s, 4H), 2.35 (br s, 6H), 1.71 (br s, 4H), 1.65 (br s, 2H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –119.32 ppm; LC–MS m/z 430 ($M + 1$).

6-Fluoro-2-((3-morpholinoethyl)amino)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (26). Prepared using general procedure C starting from **5** ($R^1 = F$) (0.3 mmol, 88 mg), yellow solid. Yield, 29% (33 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.69–7.65 (m, 2H), 7.31 (ddd, J = 2.9, 8.2, 9.1 Hz, 1H), 6.91–6.89 (m, 1H), 6.76 (s, 1H), 3.74 (dd, J = 4.6, 4.6 Hz, 4H), 3.65–3.53 (m, 4H), 2.75 (t, J = 5.9 Hz, 2H), 2.66–2.55 (m, 6H), 2.54–2.48 (m, 4H), 1.81–1.79 (m, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –118.87 ppm; LC–MS m/z 416 ($M + 1$).

6-Chloro-2-((4-morpholinobutyl)amino)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (27). Prepared using general

procedure C starting from **5** ($R^1 = \text{Cl}$) (0.3 mmol, 100 mg), white solid. Yield, 54% (50 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.97 (d, $J = 20.4$ Hz, 1H), 7.61 (d, $J = 9.0$ Hz, 1H), 7.49 (dd, $J = 2.4, 9.0$ Hz, 1H), 6.82 (s, 1H), 6.66 (s, 1H), 3.75 (t, $J = 4.7$ Hz, 4H), 3.68–3.63 (m, 2H), 3.44 (q, $J = 6.2$ Hz, 2H), 2.78 (t, $J = 5.9$ Hz, 2H), 2.62 (br s, 4H), 2.48–2.39 (m, 8H), 1.84–1.79 (m, 4H), 1.73–1.61 (m, 2H) ppm; LC–MS m/z 460 ($M + 1$).

6-Fluoro-2-((2-(piperidin-1-yl)amino)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (28). Prepared using general procedure C starting from **5** ($R^1 = \text{F}$) (0.26 mmol, 84 mg), off white solid. Yield, 9% (10 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.61–7.57 (m, 2H), 7.26 (ddd, $J = 3.0, 8.6, 8.6$ Hz, 1H), 6.70 (s, 1H), 6.67 (s, 1H), 5.44–5.42 (m, 1H), 3.57–3.44 (m, 4H), 2.66 (dd, $J = 6.0, 6.0$ Hz, 2H), 2.53–2.47 (m, 4H), 2.44–2.28 (m, 6H), 1.74–1.69 (m, 4H), 1.54–1.45 (m, 5H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –119.16 ppm; LC–MS m/z 414 ($M + 1$).

6-Fluoro-N-(2-(pyrrolidin-1-yl)ethyl)-2-((2-(tetrahydro-2H-pyran-4-yl)ethyl)amino)quinoline-4-carboxamide (29). Prepared using general procedure C starting from **5** ($R^1 = \text{F}$) (0.32 mmol, 100 mg), white solid. Yield, 7% (6 mg); ^1H NMR (500 MHz, CDCl_3) δ 7.61–7.55 (m, 2H), 7.23 (ddd, $J = 2.4, 7.8, 9.7$ Hz, 1H), 6.75 (br s, 1H), 6.59 (s, 1H), 4.74 (br s, 1H), 3.89 (dd, $J = 3.8, 11.2$ Hz, 2H), 3.55 (q, $J = 5.6$ Hz, 2H), 3.39–3.27 (m, 4H), 2.68 (t, $J = 5.9$ Hz, 2H), 2.50 (d, $J = 5.4$ Hz, 4H), 1.74–1.69 (m, 4H), 1.62–1.48 (m, 5H), 1.33–1.21 (m, 2H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –118.84 ppm; LC–MS m/z 415 ($M + 1$).

6-Chloro-N-(2-(pyrrolidin-1-yl)ethyl)-2-((2-(tetrahydro-2H-pyran-4-yl)ethyl)amino)quinoline-4-carboxamide (30). Prepared using general procedure C starting from **5** ($R^1 = \text{Cl}$) (1.5 mmol, 500 mg), yellow solid. Yield, 31% (200 mg); ^1H NMR (500 MHz, CDCl_3) δ 8.06 (br s, 1H), 8.05 (d, $J = 2.0, 4$ Hz, 1H), 7.51 (d, $J = 9.0$ Hz, 1H), 7.37 (dd, $J = 2.4, 8.8$ Hz, 1H), 7.17 (m, 1H), 4.94 (t, $J = 5.0$ Hz, 1H), 3.88 (dd, $J = 3.8, 11.2$ Hz, 2H), 3.74 (q, $J = 5.4$ Hz, 2H), 3.46 (q, $J = 6.6$ Hz, 2H), 3.33–3.27 (m, 2H), 3.16–3.06 (m, 6H), 2.03–1.98 (m, 3H), 1.63–1.49 (m, 6H), 1.32–1.21 (m, 2H) ppm; LC–MS m/z 431 ($M + 1$).

General Procedure D: Suzuki Coupling on Intermediate 5.

To a solution of **5** (1 equiv) in DMF/water 3/1 (4 mL) in a microwave vial were added potassium phosphate (3 equiv), the corresponding boronic acid (3 equiv), and $\text{Pd}(\text{PPh}_3)_4$ (3 mol %). The reaction was degassed by bubbling nitrogen through the mixture for 5 min and then heated under microwave irradiation at 130 °C for 30 min. Reaction mixture was filtered through Celite. Celite was washed with DCM. Filtrate was partitioned between water (5 mL) and DCM (25 mL \times 2). Organic phase was dried over MgSO_4 , and solvents were evaporated under reduced pressure. Product was purified by column chromatography on 4 g silica cartridges using A (DCM) and B (20% MeOH– NH_3 in DCM) and the following gradient: 3 min hold 100% A, 15 min ramp to 30% B, 4 min hold at 30% B.

2-(4-(Dimethylamino)phenyl)-6-fluoro-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (36). Prepared using general procedure D starting from **5** ($R^1 = \text{F}$) (0.2 mmol, 65 mg), yellow solid. Yield, 25% (20 mg); ^1H NMR (500 MHz, CDCl_3) δ 8.13–8.07 (m, 3H), 7.91–7.87 (m, 2H), 7.47 (ddd, $J = 2.9, 8.1, 9.2$ Hz, 1H), 6.96 (s, 1H), 6.85–6.82 (m, 2H), 3.70 (q, $J = 5.7$ Hz, 2H), 3.08 (s, 6H), 2.83 (t, $J = 6.0$ Hz, 2H), 2.67–2.64 (m, 4H), 1.86–1.81 (m, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –113.18 ppm; LC–MS m/z 407 ($M + \text{H}$) $^+$.

6-Fluoro-2-(4-morpholinophenyl)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (37). Prepared using general procedure D starting from **5** ($R^1 = \text{F}$) (0.5 mmol, 150 mg), yellow solid. Yield, 19% (43 mg); ^1H NMR (500 MHz, CDCl_3) δ 8.15–8.07 (m, 3H), 7.91–7.87 (m, 2H), 7.52–7.47 (m, 1H), 7.03 (d, $J = 8.8$ Hz, 2H), 6.89 (t, $J = 4.5$ Hz, 1H), 3.92 (t, $J = 4.8$ Hz, 4H), 3.68 (q, $J = 5.7$ Hz, 2H), 3.30 (t, $J = 4.8$ Hz, 4H), 2.79 (t, $J = 6.0$ Hz, 2H), 2.60–2.62 (m, 4H), 1.81 (t, $J = 3.3$ Hz, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –112.39 ppm; LC–MS m/z 449 ($M + \text{H}$) $^+$.

6-Fluoro-2-(4-(morpholinomethyl)phenyl)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (38). Prepared using general procedure D starting from **5** ($R^1 = \text{F}$) (0.23 mmol, 75 mg), white solid.

Yield, 20% (24 mg); ^1H NMR (500 MHz, CDCl_3) δ 8.36–8.33 (m, 2H), 8.22 (dd, $J = 5.4, 9.2$ Hz, 1H), 8.04–7.99 (m, 2H), 7.91 (d, $J = 8.5$ Hz, 2H), 7.58 (ddd, $J = 2.8, 8.0, 9.2$ Hz, 1H), 6.98 (s, 1H), 3.79–3.69 (m, 6H), 3.07 (t, $J = 4.7$ Hz, 4H), 2.81 (t, $J = 5.9$ Hz, 2H), 2.62 (d, $J = 5.4$ Hz, 4H), 1.84–1.79 (m, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –109.76 ppm; LC–MS m/z 513 ($M + \text{H}$) $^+$.

6-Fluoro-2-(4-(morpholine-4-carbonyl)phenyl)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (39). Prepared using general procedure D starting from **5** ($R^1 = \text{F}$) (0.25 mmol, 80 mg), yellow solid. Yield, 73% (87 mg); ^1H NMR (500 MHz, CDCl_3) δ 8.18–8.14 (m, 3H), 7.96–7.94 (m, 2H), 7.54–7.50 (m, 3H), 7.10 (dd, $J = 4.9, 4.9$ Hz, 1H), 3.76–3.72 (m, 6H), 3.69–3.66 (m, 4H), 2.78 (t, $J = 6.0$ Hz, 2H), 2.61–2.59 (m, 4H), 1.81–1.79 (m, 4H) ppm; ^{19}F NMR (407.5 MHz; CDCl_3) δ –110.76 ppm; LC–MS m/z 477 ($M + \text{H}$) $^+$.

1-(4-(Bromomethyl)-2-chlorophenyl)ethanone (7). A mixture of 1-(2-chloro-4-methylphenyl)ethanone (1.6 g, 9.50 mmol) and chlorobenzene (60 mL) was prepared at rt and *N*-bromosuccinimide (NBS) (1.86 g, 10.44 mmol) added followed by benzoyl peroxide (~1.5 mg, 0.005 mmol, catalytic amount), and the mixture was heated to 140–145 °C for 16 h. The mixture was then cooled to rt, diluted with toluene (50 mL), and filtered through a Celite pad. The pad was washed with toluene (2 \times 50 mL) and the filtrate concentrated under reduced pressure and purified by column chromatography (0–10% EtOAc/hexanes) to afford 1-(4-(bromomethyl)-2-chlorophenyl)ethanone (1.65 g, 6.65 mmol, 70%) as a yellow oil. ^1H NMR (500 MHz; CDCl_3) δ 2.65 (s, 3H), 4.43 (s, 2H), 7.34 (dd, 1H, $J = 1.3, 8.0$ Hz), 7.46 (s, 1H), 7.54 (d, 1H, $J = 7.9$ Hz) ppm.

1-(2-Chloro-4-(morpholinomethyl)phenyl)ethanone (8, X = Cl). A mixture of 1-(4-(bromomethyl)-2-chlorophenyl)ethanone (1.65 g, 6.65 mmol) and acetonitrile (25 mL) was prepared at rt and stirred under nitrogen. Potassium carbonate (1.10 g, 7.98 mmol) was then added followed by morpholine (0.695 mL, 6.95 mmol, 7.98 mmol), and the mixture was stirred at rt. After 2 h, TLC showed presence of product and starting material. Mixture was then heated under nitrogen to 40 °C for 16 h, cooled to room temp, filtered to remove excess carbonate and filtrate concentrated under reduced pressure. Mixture was then diluted in DCM (30 mL), washed with water (2 \times 10 mL), filtered through a phase separator and filtrate concentrated under reduced pressure. Mixture was purified by column chromatography (40–100% ethyl acetate/hexane) to afford 1-(2-chloro-4-(morpholinomethyl)phenyl)ethanone (1.08 g, 4.25 mmol, 64%) as a yellow oil. ^1H NMR (500 MHz; CDCl_3) δ 2.44 (brs, 4H), 2.65 (s, 3H), 3.49 (s, 2H), 3.72 (t, $J = 4.6$ Hz, 4H), 7.29 (dd, $J = 1.5, 7.9$ Hz, 1H), 7.43 (brs, 1H), 7.55 (d, $J = 7.9$ Hz, 1H) ppm; LCMS m/z 254 [$M + \text{H}$] $^+$.

2-(2-Chloro-4-(morpholinomethyl)phenyl)-6-fluoroquinoline-4-carboxylic Acid (9, X = Cl). A mixture of 5-fluoroisatin (7.02 mg, 4.25 mmol) and 1-(2-chloro-4-(morpholinomethyl)phenyl)ethanone (1.08 g, 4.25 mmol) was prepared in EtOH/water (1:1) (10 mL) and then KOH (2.40 g, 42.50 mmol) added and the mixture heated in microwave, 125 °C, 20 min. The mixture was then diluted with water (10 mL), acidified to pH 3 with 2 M HCl, stirred for 16 h at rt and the resulting precipitate filtered, washed with water (2 \times 10 mL), and concentrated under reduced pressure to afford 2-(2-chloro-4-(morpholinomethyl)phenyl)-6-fluoroquinoline-4-carboxylic acid (503 mg, 1.25 mmol, 30%) as an orange solid. ^1H NMR (500 MHz; CDCl_3) δ 2.54 (brs, 4H), 3.64 (s, 4H), 3.70 (brs, 2H), 7.50 (d, $J = 8.1$ Hz, 1H), 7.62 (s, 1H), 7.73 (bd, $J = 7.9$ Hz, 1H), 7.84 (dt, $J = 2.9, 8.2$ Hz, 1H), 8.24 (dd, $J = 5.8, 9.2$ Hz, 1H), 8.56 (dd, $J = 2.9, 11.0$ Hz, 1H) ppm; LCMS m/z 399 [$M - \text{H}$] $^-$.

2-(2-Chloro-4-(morpholinomethyl)phenyl)-6-fluoro-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (42). A mixture of 2-(2-chloro-4-(morpholinomethyl)phenyl)-6-fluoroquinoline-4-carboxylic acid (303 mg, 0.76 mmol) in DCM (6 mL) was prepared at rt, and *N*-methylmorpholine (0.166 mL, 153 mg, 1.51 mmol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (159 mg, 0.91 mmol) were added, and the mixture was stirred for 1 h in a sealed vial. 2-(Pyrrolidin-1-yl)ethanamine (0.143 mL, 130 mg, 1.13 mmol) was then added and the mixture stirred in a sealed vial for 17 h. The mixture was

then diluted with DCM (5 mL), and the organic layers were washed with water (2 × 3 mL) and filtered through a phase separator and the organic layers concentrated under reduced pressure and purified by column chromatography (0–10% 7 M NH₃ in MeOH/DCM) to afford an off white solid. Analysis by ¹H NMR showed impurities, and the mixture was further purified by MDAP to produce 2-(2-chloro-4-(morpholinomethyl)phenyl)-6-fluoro-*N*-(2-(pyrrolidin-1-yl)ethyl)-quinoline-4-carboxamide (228 mg, 0.46 mmol, 61%) as an off-white solid. ¹H NMR (500 MHz; CDCl₃) δ 1.76–1.79 (m, 4H), 2.49 (brs, 4H), 2.57 (brs, 4H), 2.75 (t, *J* = 6.0 Hz, 2H), 3.55 (s, 2H), 3.65 (q, *J* = 5.3 Hz, 2H), 3.74 (t, *J* = 4.6 Hz, 4H), 6.82 (brs, 1H), 7.40 (dd, *J* = 1.6, 7.9 Hz, 1H), 7.52–7.57 (m, 2H), 7.68 (d, *J* = 7.9 Hz, 1H), 7.89 (s, 1H), 8.05 (dd, *J* = 2.8, 10.0 Hz, 1H), 8.19 (dd, *J* = 5.5, 9.2 Hz, 1H) ppm; LCMS *m/z* 497 [M + H]⁺.

6-Fluoro-*N*-(2-morpholinoethyl)-2-(4-morpholinomethyl)-phenylquinoline-4-carboxamide (46). A solution of 6-fluoro-2-(4-(morpholinomethyl)phenyl)quinoline-4-carboxylic acid (9, X = H)⁵ (0.1 g, 0.27 mmol), 2-chloro-4,6-dimethoxy-1,3,5-triazine CDMT (57 mg, 0.32 mmol, 1.2 equiv), and *N*-methylmorpholine (0.06 mL, 0.54 mmol, 2 equiv) in DCM (10 mL) was stirred at room temperature for 1 h. 2-Morpholinoethanamide (0.054 mL, 0.41 mmol, 1.5 equiv) was added, and the reaction mixture was stirred at room temperature overnight. Reaction was partitioned between DCM (50 mL) and NaHCO₃ sat. aq solution (10 mL). Organic phase was dried over MgSO₄, and solvents were removed under reduce pressure. Product precipitated from acetonitrile (1.5 mL) as a white solid. Yield, 63% (83 mg); ¹H NMR (500 MHz, CDCl₃) δ 8.22 (dd, *J* = 5.4, 9.2 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 2H), 8.00–7.97 (m, 2H), 7.58–7.52 (m, 3H), 6.67 (t, *J* = 4.8 Hz, 1H), 3.77–3.70 (m, 10H), 3.61 (s, 2H), 2.70 (t, *J* = 5.9 Hz, 2H), 2.57 (br s, 4H), 2.51 (t, *J* = 4.3 Hz, 4H) ppm; ¹⁹F NMR (407.5 MHz; CDCl₃) δ –111.23; LC–MS *m/z* 479 (M + H)⁺.

6-Fluoro-*N*-methyl-2-(4-(morpholinomethyl)phenyl)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (47). To a suspension of 6-fluoro-2-[4-(morpholinomethyl)phenyl]quinoline-4-carboxylic acid (9, X = H)⁵ (100 mg, 0.27 mmol) in anhydrous DCM (10 mL) were added *N*-methylmorpholine (0.06 mL, 0.54 mmol, 2 equiv) and 2-chloro-4,6-dimethoxy-1,3,5-triazine CDMT (57 mg, 0.32 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 1 h. *N*-Methyl-2-pyrrolidin-1-ylethanamine (0.034 g, 0.27 mmol) was added and the reaction mixture stirred at room temperature overnight. The mixture was then diluted with DCM (10 mL), water (10 mL) was added, and the layers were separated. The aqueous portion was extracted with further DCM (10 mL). The combined DCM extracts were evaporated in vacuo. The residue was dissolved in DMF and purified by mass directed autoprep 5–95% MeCN, basic, to afford impure product. The sample was dissolved in DMF and purified using mass directed autoprep 25–75% MeCN, basic, and the product obtained was freeze-dried to afford 47 as a cream colored solid (24 mg, 17% yield); ¹H NMR (500 MHz, CDCl₃) δ 8.15–8.08 (m, 3H), 8.04–8.01 (m, 1H), 7.48–7.42 (m, 3H), 7.36 (dd, *J* = 2.8, 9.2 Hz, 1H), 4.06–4.03 (m, 1H), 3.67 (dd, *J* = 4.3, 4.3 Hz, 4H), 3.53 (s, 2H), 3.27 (s, 2H), 3.22 (s, 1H), 2.90 (s, 3H), 2.43 (s, 4H), 2.11–2.06 (m, 4H), 1.81–1.51 (m, 4H) ppm; LC–MS *m/z* 477 (M + H)⁺.

***N*-(2-(Cyclopropylamino)ethyl)-6-fluoro-2-(4-(morpholinomethyl)phenyl)quinoline-4-carboxamide (48).** To a suspension of 6-fluoro-2-[4-(morpholinomethyl)phenyl]quinoline-4-carboxylic acid (9, X = H)⁵ (0.1 g, 0.27 mmol) in anhydrous DCM (10 mL) were added *N*-methylmorpholine (0.06 mL, 0.54 mmol, 2 equiv) and 2-chloro-4,6-dimethoxy-1,3,5-triazine CDMT (57 mg, 0.32 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 1 h. *N'*-Cyclopropylethane-1,2-diamine (0.027 g, 0.27 mmol) was added and the reaction mixture stirred at room temperature for 2.5 days. The mixture was then diluted with DCM (10 mL), water (10 mL) was added, and the layers were separated. The aqueous portion was extracted with further DCM (10 mL). The combined DCM extracts were evaporated in vacuo. The residue was dissolved in DMF and purified by mass directed autoprep 5–95% MeCN, basic, method to afford 48 as a yellow solid (25 mg, 18% yield); ¹H NMR (500 MHz, CDCl₃) δ 8.18 (dd, *J* = 5.4, 9.2 Hz, 1H), 8.09 (d, *J* = 8.2 Hz, 2H), 7.94–7.91 (m, 2H), 7.55–7.49 (m, 3H), 6.74–6.72 (m, 1H), 3.75–

3.66 (m, 6H), 3.59 (s, 2H), 3.06 (t, *J* = 5.8 Hz, 2H), 2.49 (s, 4H), 2.23–2.18 (m, 1H), 0.54–0.49 (m, 2H), 0.39–0.35 (m, 2H); LC–MS *m/z* 449 (M + H)⁺.

***N*-(2-(Azetidin-1-yl)ethyl)-6-fluoro-2-(4-(morpholinomethyl)-phenyl)quinoline-4-carboxamide (49).** To a suspension of 6-fluoro-2-[4-(morpholinomethyl)phenyl]quinoline-4-carboxylic acid (9, X = H)⁵ (100 mg, 0.27 mmol) in anhydrous DCM (10 mL) were added *N*-methylmorpholine (0.06 mL, 0.54 mmol, 2 equiv) and 2-chloro-4,6-dimethoxy-1,3,5-triazine CDMT (57 mg, 0.32 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 1 h. 2,2-Dimethoxyethanamine (28 mg, 0.27 mmol) was added and the reaction mixture stirred at room temperature for 2.5 days. The mixture was then diluted with DCM (10 mL), water (10 mL) was added, and the layers were separated. The aqueous portion was extracted with further DCM (10 mL). The combined DCM extracts were evaporated in vacuo. The residue was dissolved in DCM and purified by silica (12 g), eluting with 0–100% EtOAc/hexane and then 0–50% (10% MeOH in DCM/DCM) to afford *N*-(2,2-dimethoxyethyl)-6-fluoro-2-[4-(morpholinomethyl)phenyl]quinoline-4-carboxamide as a yellow gum (109 mg, 79% yield). A solution of *N*-(2,2-dimethoxyethyl)-6-fluoro-2-[4-(morpholinomethyl)phenyl]quinoline-4-carboxamide (109 mg, 0.24 mmol) in 1,4-dioxane (5 mL) was treated with conc. HCl (1 mL) and stirred at room temperature for 1.5 h. The mixture was neutralized with saturated sodium bicarbonate portionwise and then extracted with EtOAc (2 × 20 mL). The combined EtOAc extracts were evaporated in vacuo to afford 6-fluoro-2-[4-(morpholinomethyl)-phenyl]-*N*-(2-oxoethyl)quinoline-4-carboxamide as a yellow gum (78 mg, 71% yield). A mixture of 6-fluoro-2-[4-(morpholinomethyl)-phenyl]-*N*-(2-oxoethyl)quinoline-4-carboxamide (78 mg, 0.19 mmol), azetidine (32 mg, 0.57 mmol) in DCM (5 mL) was stirred for 15 min in a stoppered flask at room temperature. Sodium triacetoxylborohydride (56 mg, 0.26 mmol) was then added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was partitioned between water (10 mL) and DCM (10 mL) and the aqueous extracted with further DCM (10 mL). The combined DCM extracts were evaporated in vacuo. The residue was dissolved in DMF and purified by mass directed autoprep, 5–95% MeCN, basic, to afford a yellow gum. The sample was freeze-dried to afford 49 as a cream colored solid (18 mg, 14% yield); ¹H NMR (500 MHz, CDCl₃) δ 8.21 (dd, *J* = 5.4, 9.2 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.00–7.97 (m, 2H), 7.58–7.52 (m, 3H), 6.73–6.72 (m, 1H), 3.76 (t, *J* = 4.6 Hz, 4H), 3.61 (s, 2H), 3.57–3.53 (m, 2H), 3.28 (t, *J* = 7.0 Hz, 4H), 2.72 (t, *J* = 5.8 Hz, 2H), 2.51 (s, 4H), 2.14–2.07 (m, 2H); LC–MS *m/z* 449 (M + H)⁺.

Details of other synthetic routes for individual compounds are described in the [Supporting Information](#).

Biology Materials and Methods. This is included in the [Supporting Information](#).

Ethical Statements. In vivo antimalarial efficacy studies in *P. berghei* carried out at the Swiss Tropical and Public Health Institute (Basel, Switzerland) adhere to local and national regulations of laboratory animal welfare in Switzerland (awarded Permission No. 1731). Protocols are regularly reviewed and revised following approval by the local authority (Veterinäramt Basel Stadt).

Mouse and rat pharmacokinetics were carried out at the University of Dundee. All regulated procedures on living animals were carried out under the authority of a license issued by the Home Office under the Animals (Scientific Procedures) Act 1986, as amended in 2012 (and in compliance with EU Directive EU/2010/63). License applications will have been approved by the University's Ethical Review Committee (ERC) before submission to the Home Office. The ERC has a general remit to develop and oversee policy on all aspects of the use of animals on University premises and is a subcommittee of the University Court, its highest governing body.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00723.

Synthetic details for all compounds, supplementary data tables, additional information on ADMET and pharmacology (PDF)

Molecular formula strings and some data (CSV)

■ AUTHOR INFORMATION

Corresponding Authors

*K.D.R.: phone, +44 1382 388 688; e-mail, k.read@dundee.ac.uk.

*I.H.G.: phone, +44 1382 386 240; e-mail, i.h.gilbert@dundee.ac.uk.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ACT, artemisinin combination therapy; CDMT, 2-chloro-4,6-dimethoxy-1,3,5-triazine; *Cl_i*, intrinsic clearance; EDC, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; HOBt, hydroxybenzotriazole; LELP, ligand-efficiency-dependent lipophilicity; LLE, lipophilic ligand efficiency; MMV, Medicines for Malaria Venture; *P_e*, permeability; TCP, target candidate profile; WHO, World Health Organization

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